IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Samuel Weiss	Art Unit	: 1636
Serial No.	: 10/523,253	Examiner	: Laura L. McGillem
Filed	: January 26, 2005	Conf. No.	: 8661
Title	: OLIGODENDROCYTE PRODUCTION FROM MULTIPOTENT NEURAL STEM CELLS		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132 BY SAMUEL WEISS

I, Samuel Weiss, Ph.D., pursuant to 37 C.F.R. § 1.132, declare the following:

1. I am the Director of the Hotchkiss Brain Institute and the Alberta Heritage Foundation for Medical Research Scientist in the Departments of Cell Biology & Anatomy and Pharmacology & Therapeutics at the University of Calgary, Faculty of Medicine, Calgary, Alberta. I have been engaged in neurobiochemistry research for over 20 years.

2. I am a co-inventor of the claimed invention described in the patent application captioned above ("the patent application").

3. I provide herein data showing the results of in vivo experiments performed in my laboratory under my direction. GM-CSF was infused into 6-week old CD1 male mice for 6 days followed by examination of the factor's effects in the corpus callosum. Immunohistochemistry with antibodies against PDGFRα and GSTπ (*see* Figure 1 at Appendix A) was used to quantify the number of oligodendrocyte progenitor cells and the total number of mature oligodendrocytes in the corpus callosum. GM-CSF increased the number of BrdU-positive cells in the corpus callosum by 17-fold ($p < 0.001$; $n = 5$) (*see* Table 1 at Appendix B). Further, GM-CSF increased the number of newly generated PDGFRα/BrdU-positive cells by 23-fold ($p < 0.001$; $n = 5$) (*see* Table 1 (Appendix B) and Figures 1 and 2 (Appendix A)). GM-CSF also increased the percentage of PDGFRα/BrdU-positive cells of the total PDGFRα-positive cells compared to

control infusions by a similar factor (*see* Table 1 at Appendix B). These results indicate that GM-CSF increased production of new oligodendrocytes. When examining the number of terminally differentiated oligodendrocytes in the corpus callosum, GM-CSF increased the number of GSTπ-positive cells by approximately 2-fold compared to control (*see* Table 1 at Appendix B), and resulted in a significantly greater number of GSTπ/BrdU-positive cells (*see* Figure 2 at Appendix A). This number of GSTπ/BrdU-positive cells was approximately 7-fold as compared to the control level ($p < 0.01$; $n = 5$) (*see* Figure 1 at Appendix A). GM-CSF also significantly increased the percent of GSTπ/BrdU-positive cells of the total number of GSTπ-positive cells by approximately 4-fold as compared to the control level ($p < 0.01$; $n = 5$) (*see* Table 1 at Appendix B). These results indicate that although GM-CSF increases the number of terminally differentiated oligodendrocytes in the corpus callosum, GM-CSF induces a significant number of newly generated oligodendrocytes to survive for extended periods of time.

4. Further, the number of apoptotic terminally differentiated oligodendrocytes in the corpus callosum following ICV infusion of GM-CSF was examined by immunostaining for the activated form of caspace-3, a marker of apoptotic cells, and GSTπ (*see* Figures 1 and 2 at Appendix A). There were significantly fewer activated caspase-3-positive cells in the corpus callosum following GM-CSF infusion compared to control ($p < 0.05$; $n = 4$ for GM-CSF animals, $n = 5$ for control animals) (*see* Table 1 at Appendix B). When examining the number of apoptotic oligodendrocytes, there were significantly fewer activated caspase-3/GSTp-positive cells following GM-CSF infusion compared to control infusions ($p < 0.05$; $n = 4$ for GM-CSF animals, $n = 5$ for control animals) (*see* Table 1 (Appendix B) and Figure 1 (Appendix A)). These data demonstrate that GM-CSF is promoting the survival of terminally differentiated oligodendrocytes *in vivo* in the corpus callosum.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: August 2, 2007

Samuel Weiss, Ph.D.

Appendix A

Figure 1. Representative images of the corpus callosum on the contralateral side of ICV infusions.

Representative fields at 25X magnification of BrdU alone (*A-B*), BrdU (green) and PDGFRα (red) (*D-E*), BrdU (green) and GSTπ (red) (*G-H*), activated caspase-3 (AC-3) (green) and GSTπ (red) (*J-K*) and BrdU (green) and Iba1 (red) (*M-N*). Scale bar, 100 μm.

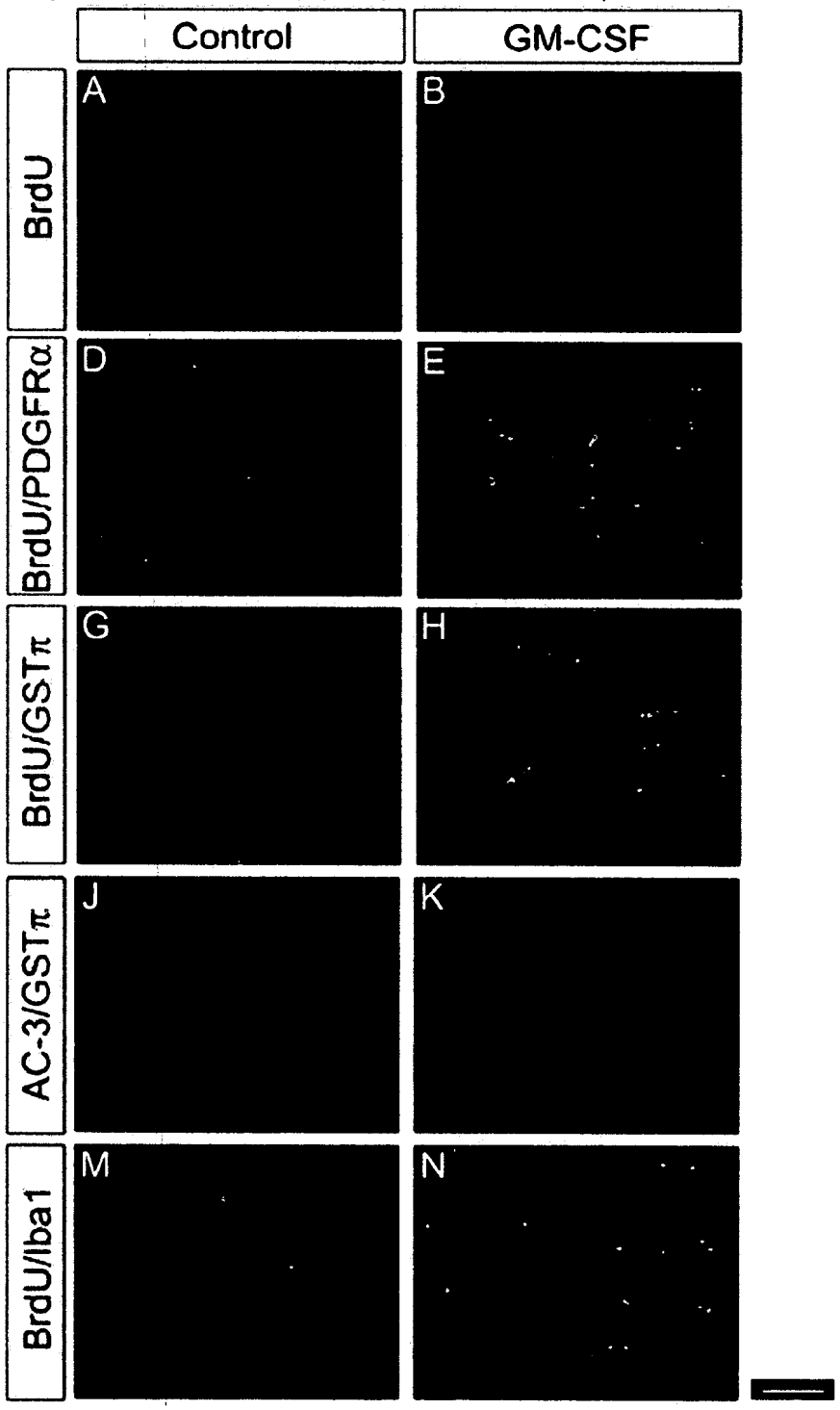
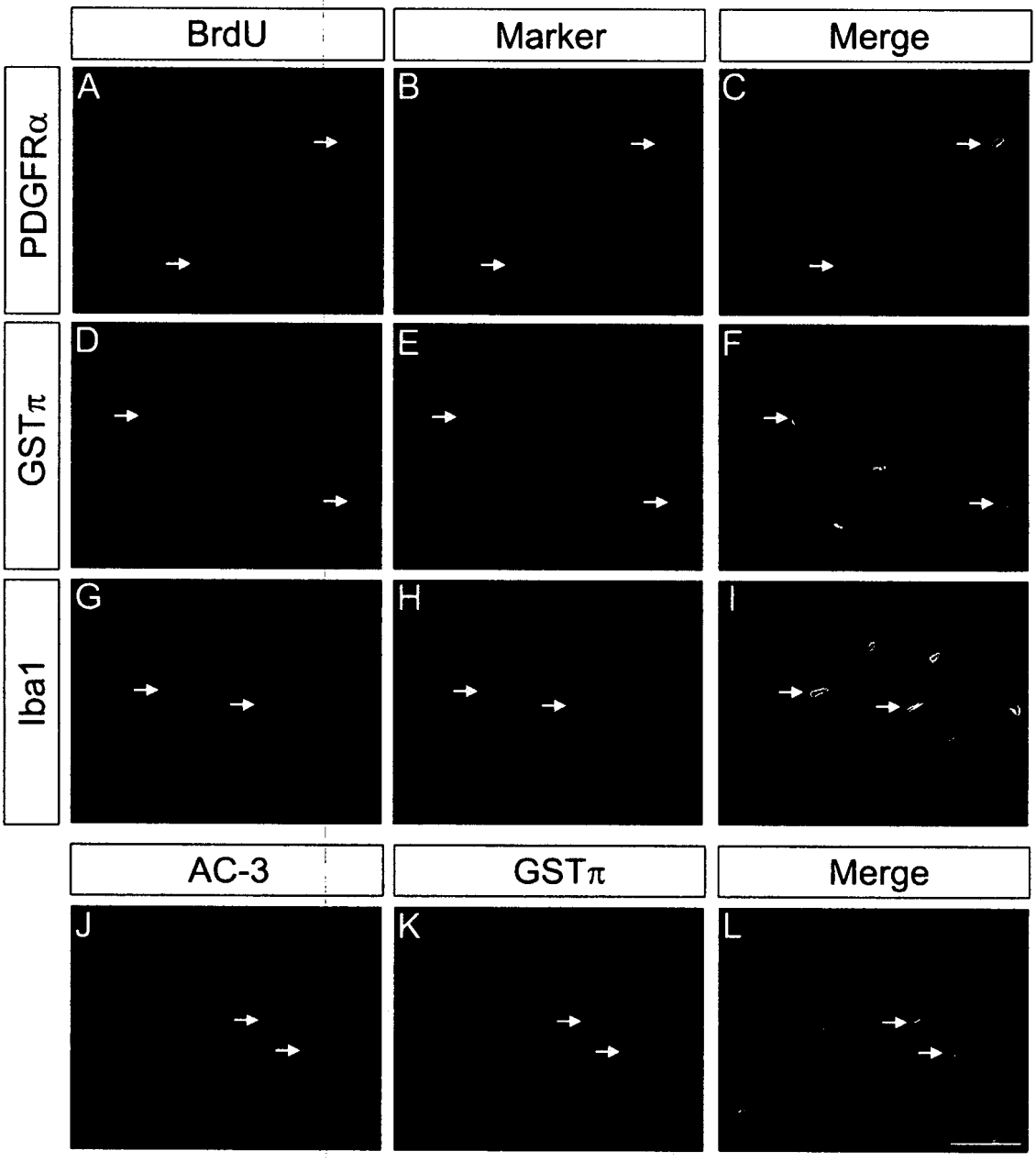


Figure 2. Representative images at higher magnification of double-labeled cells in the corpus callosum of ICV infusions.
Arrows point to double-labeled BrdU and PDGFRα (*A-C*), BrdU and GSTπ (*D-F*), BrdU and Iba1 (*G-I*) and activated caspase-3 (AC-3) and GSTπ (*J-L*) cells at 63X magnification. Scale bar, 50 μm.



Appendix B

Table 1. Oligodendroglial and microglial production on the contralateral side of the corpus callosum following growth factor ICV infusion

	Infusion	
Cell Marker	Control	GM-CSF
BrdU	124.2 ± 22.4	2174.8 ± 245.4***
PDGFRα	359.6 ± 31.6	1215.2 ± 81.3***
PDGFRα/BrdU	11.2 ± 2.4	263.0 ± 54.7***
% PDGFRα/BrdU (of total PDGFRα cells)	3.1 ± 0.7	20.9 ± 3.2***
GSTπ	1787.8 ± 225.0	3876.8 ± 158.6***
GSTπ/BrdU	22.0 ± 5.1	166.0 ± 37.1**
% GSTπ/BrdU (of total GSTπ cells)	1.2 ± 0.2	4.2 ± 0.9**
Activated Caspase-3	202.4 ± 31.0	77.0 ± 6.0*
GSTπ/Activated Caspase-3	79.8 ± 10.2	9.0 ± 2.0*

Adult CD1 males were infused for 6 days, perfused and their brains analyzed through immunohistochemistry with the corresponding cell markers for oligodendrocytes and microglia. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ from control; $n = 5$ for each infusion condition, except activated caspase-3 where $n = 4$.